

Interplay between mutational pathway and drug gradient controls time to evolution of drug resistance

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Drug gradients are believed to play an important role in the evolution of drug resistance, from antibiotics to cancer. We use a statistical physics model to study the evolution of a population of malignant cells exposed to drug gradients, where drug resistance emerges via multiple mutations. We show that a non-uniform drug concentration can strongly accelerate the emergence of resistance when the mutational pathway involves a long sequence of mutants with increasing resistance, but slows it down if the pathway is short or crosses a fitness valley. These predictions can be verified experimentally, and have the potential to improve strategies to combat the emergence of resistance.

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The evolution of drug resistance is an urgent problem in the treatment of diseases, from bacterial infections to cancer. Attempts to address this problem include the characterization of mutational pathways leading to resistance [1, 2], as well as theoretical [3–8] and experimental [9–11] studies of the emergence of resistance under different treatment regimens. These studies often assume a spatially uniform drug concentration. However, in many clinical situations drug concentrations vary in space [12, 13], for example where malignant cells form less drug-permeable layers such as bacterial biofilms [14] or tumour stromas [15]. Recent experimental work [16] suggests that the evolution of antibiotic resistance in bacterial populations can be greatly accelerated if the antibiotic concentration is spatially non-uniform.

It is often observed that several mutations are required to obtain maximal resistance to a drug [1, 2, 16]. In some cases, fitness (i.e. drug resistance) increases steadily along the mutational pathway to full resistance [1]; in other cases, epistatic interactions between mutations may result in less fit intermediate genotypes (fitness “valleys”) [17–19]. The role of mutational pathways in controlling evolutionary dynamics has been studied in the quasispecies model [20, 21] and in models of cancer progression [22, 23]. That work, however, does not take into account the effects of spatial structure. On the other hand, in models without complex evolutionary pathways, it is well known that spatial structure can increase genetic diversity, the rate of evolutionary diversification [24–28], and the rate of viral drug resistance [29]; indeed, in a broader statistical physics context, spatial structure plays a key role in many theoretical studies of evolving populations [24, 30–35].

Here we use a statistical physics model to show that, in the presence of drug gradients, a population evolves drug resistance in a sequence of waves of increasingly better adapted mutants that extend its range in a step-wise manner. In contrast, for a uniformly distributed

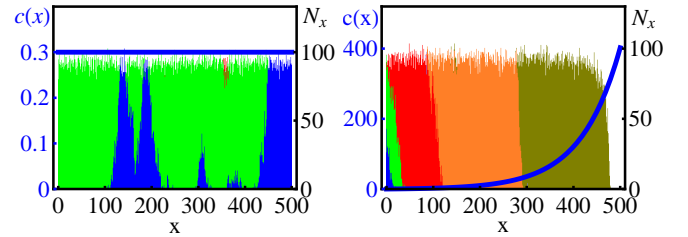


Figure 1: Simulation snapshots for the cases of a uniform and an exponentially increasing drug concentration (left and right, respectively). Blue lines show the drug concentration (left axes), while the colors represent the populations of the different genotypes (right axes). Parameter values are $K = 100$, $L = 500$, $M = 6$, $\mu = 5 \times 10^{-6}$, $c_m^{\text{mic}} = 4^{m-1}$, and the drug concentration $c = 0.3$ (left panel) and $c(x) = e^{\alpha x} - 1$ with $\alpha = 0.06$ (right panel). For corresponding movies see [36].

drug, resistant mutants evolve at random positions and spread over the entire environment. The rate of evolution depends crucially on the mutational pathway leading to drug resistance. If tolerance to the drug increases monotonously along the pathway, drug gradients can significantly accelerate the evolution by increasing selection at the population’s edge. However, if the pathway crosses a fitness valley, evolution of resistance may actually be slowed down by a non-uniform drug concentration, as a result of a reduced rate of “stochastic tunnelling” due to a smaller population size. We also suggest how our predictions could be verified experimentally and used to infer the structure of the mutational pathway to resistance.

The model. We consider a growing population of cells which mutate between M possible genotypes, with different levels of resistance to a drug. To model the effects of spatial heterogeneity, the population is assumed to reside within a chain of L connected microhabitats, which may contain different concentrations of the drug. These discrete microhabitats might represent connected chambers

in a microfluidic experiment [16]; in the limit of small microhabitats, our model represents a population growing in continuous space. Within a given microhabitat i the population is assumed to be well-mixed, with a fixed carrying capacity K ; cells of genotype m replicate at rate $\phi_m(c_i)(1 - N_i/K)$ where N_i is the total population of cells in microhabitat i , c_i is the drug concentration and $\phi_m(c_i)$ is the growth rate of genotype m , which depends on the local drug concentration. Upon replication, cells mutate with probability μ ; we shall mainly consider the case of an unbranched mutational pathway to drug resistance, such that genotype m mutates only into genotypes $m \pm 1$ (without any bias). Cells migrate between microhabitats i and $i \pm 1$ at rate $b/2$ and die at a fixed rate d .

A key feature of our model is the fact that different genotypes show different levels of drug resistance: genotype 1 is least resistant while genotype M is most resistant. The *minimal inhibitory concentration* (MIC) c_m^{mic} denotes the drug concentration at which genotype m ceases to be able to grow; this is embodied in our model in the assumption that $\phi_m(c) = \max \left\{ 0, 1 - (c/c_m^{\text{mic}})^2 \right\}$. This choice is inspired by Ref. [37] (see also [38]).

We study this model using kinetic Monte Carlo simulations [39] which are initiated with $N_1 = K$ cells of genotype 1 in microhabitat 1 and with all the other microhabitats empty, so that the population colonizes the space during the simulation. We define the units of time in our simulations by fixing the maximal growth rate $\phi_m(0) = 1$ and the units of drug concentration by fixing $c_1^{\text{mic}} = 1$, and we set $b = 0.1$, $d = 0.1$, and $K = 100$ (see [38] for a detailed discussion of the choice of the parameters). We use values of μ and L such that the number of mutants per generation emerging anywhere in the environment is typically small, $\mu KL \leq 1$ (see figure captions for details). Our results are thus relevant for not-too-large populations, for which stochastic effects play an important role.

Monotonically increasing MIC. We first consider a pathway to resistance for which the M genotypes have increasing levels of drug resistance – i.e. $c_m^{\text{mic}} > c_{m-1}^{\text{mic}}$ for all $m > 1$, as depicted in Fig. 2c. In particular, we set $M = 6$ and $c_m^{\text{mic}} = 4^{m-1}$; the ratio $c_6^{\text{mic}}/c_1^{\text{mic}} \approx 10^3$ between fully resistant and wild-type cells is consistent with experimentally determined values [1] (see also [38]). We compare the “homogeneous” case where the drug concentration is uniform ($c_i \equiv c$) with the “spatially heterogeneous” case where the drug concentration $c_i = \exp(\alpha i) - 1$ is non-uniform and increases exponentially from left to right with steepness α (see solid lines in Fig. 1).

The emergence of drug resistance occurs very differently in the homogeneous and heterogeneous cases, as illustrated in the simulation snapshots of Fig. 1. In the homogeneous case (Fig. 1 left), genotype 1 (blue) first spreads to fill the entire space, then mutants of genotype 2 (green) emerge at random locations; these spread

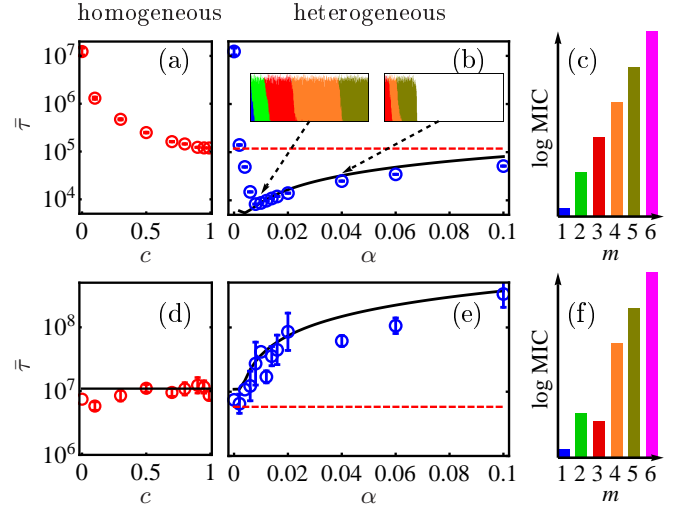


Figure 2: Average time to resistance $\bar{\tau}$ for homogeneous ((a,d), red circles) and heterogeneous ((b,e), blue circles) drug concentrations, for $M = 6$, $L = 500$, $K = 100$, and $\mu = 5 \times 10^{-6}$. Panels (a,d): exponentially increasing MIC (c), panels (b,e): fitness valley (f). For the heterogeneous case (b,e), the red dashed lines show the minimal value of $\bar{\tau}$ obtained for the homogeneous case (i.e. the minimum of $\bar{\tau}(c)$ from (a,d)). The black lines show the theoretical predictions of Eqs. (3) (exponential MIC) and Eqs. (4), (5) (valley MIC). The insets show simulation snapshots taken just before the first occurrence of genotype $m = 6$, for two values of α (indicated by arrows).

because they have a growth advantage in the drug environment, before giving rise to genotype 3 (red), etc. [47]. In contrast, in the heterogeneous case, waves of increasingly better-adapted mutants invade the environment from left to right in a step-wise manner. Each genotype colonizes the space only up to a well-defined spatial boundary, where the local drug concentration approaches its MIC and the population forms a stationary “front”. Better-adapted mutants then emerge at the edge of this front to further colonize the space.

To quantify the effect of a spatial drug distribution on the emergence of drug resistance, we plot in Fig. 2a,b the mean time $\bar{\tau}$ to emergence of full drug resistance – i.e. the time to emergence of a mutant with $m = M = 6$, averaged over surviving populations. For a homogeneous drug concentration $c_i = c$, $\bar{\tau}$ decreases as the drug concentration c increases (Fig. 2a). Resistance emerges fastest when c approaches the MIC of genotype 1 (defined to be unity). For drug concentrations above c_1^{mic} , however, the population does not evolve resistance because the initial genotype cannot reproduce. For the non-uniform drug concentration (Fig. 2b), $\bar{\tau}$ varies non-monotonically as a function of the steepness α , with a minimum at $\alpha \approx 0.01$. This minimum arises because for very small α , little drug is present, reducing the selection pressure for the evolution of resistant mutants, whereas

for very large α , the fronts become narrow, reducing the size of the “zone” in which new resistant mutants can emerge (see snapshots in Fig. 2b).

Importantly, for almost all values of α and c , resistance emerges faster for the non-uniform drug concentration than for the uniform case; the minimal value of $\tau(\alpha)$ in the non-uniform case is smaller by an order of magnitude than the minimal value of $\tau(c)$ in the uniform case (dashed line in Fig. 2b). Thus, if the MIC increases monotonically along the pathway to resistance, a non-uniform drug concentration carries the potential for much faster evolution of drug resistance than is possible in a uniform drug concentration.

These results can be rationalized using simple physical arguments. For the non-uniform drug concentration we consider separately the colonization of space by a subpopulation of cells of genotype m and the stochastic emergence of mutants of genotype $m + 1$ within this subpopulation. In the continuous approximation (valid for large L and $\alpha \ll 1$) and assuming a unit distance between the habitats, the expansion of a wave of mutants of genotype m is described by the Fisher-KPP equation [40]:

$$\begin{aligned} \partial_t N_m &= \frac{b}{2} \partial_{xx} N_m + \phi_m N_m \left(1 - \frac{N_m}{K}\right) - d N_m \\ &= \frac{b}{2} \partial_{xx} N_m + (\phi_m - d) N_m \left[1 - \frac{\phi_m N_m}{K(\phi_m - d)}\right], \end{aligned} \quad (1)$$

where $x \equiv i$, $\phi_m \equiv \phi_m(c(x))$ and $N_m \equiv N_m(x, t)$ denotes the population of genotype m . If $c(x) \ll c_m^{\text{mic}}$, $\phi_m \approx 1$ and Eq. (1) describes a Fisher wave propagating with speed $v \approx \sqrt{2b(1-d)}$ [40]. The wave stops when it reaches the point where $c(x) \approx c_m^{\text{mic}}$; for small b the stationary solution of Eq. (1) reads $N_m^*(x) = K[1 - d/\phi_m(c(x))]$, which decays to zero at $x_m^* = (1/\alpha) \ln(c_m^{\text{mic}} \sqrt{(1-d)} + 1)$. Assuming that the wave of mutants of genotype m emerges at x_{m-1}^* (i.e. at the stationary front of the preceding wave), the time it takes to reach its stationary state is then $T_m^{\text{wave}} \approx (x_m^* - x_{m-1}^*)/v$, with $T_1^{\text{wave}} \approx x_1^*/v$.

Once the stationary population of genotype m is established, the waiting time before a new wave of mutants of genotype $m + 1$ arises can be expressed for low mutation rates as the inverse of the total rate at which mutants establish in the population,

$$T_{m+1}^{\text{mut}} = \left[\frac{\mu}{2} \int_{x_{m-1}^*}^{x_m^*} N_m^*(x) r(x) P_{\text{fix}}(x) dx \right]^{-1}. \quad (2)$$

Here $\mu/2$ is the probability to mutate from genotype m to $m + 1$, $r(x) \approx d$ is the rate of reproduction in the steady state and $P_{\text{fix}} = (\phi_{m+1} - \phi_m)/\phi_{m+1}$ is the probability of fixation of genotype $m + 1$; this is a standard result [41].

The mean total time until the first cell of genotype M

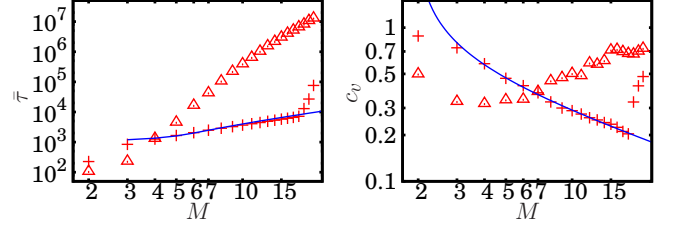


Figure 3: Average time $\bar{\tau}$ to full resistance (left) and its coefficient of variation $c_v = \sqrt{\tau^2 - \bar{\tau}^2}/\bar{\tau}$ (right) as a function of the mutational pathway length M for homogeneous ($c = 0.9$; triangles) and heterogeneous ($\alpha = 0.07$; pluses) drug concentration. In both cases $L = 300$, $K = 100$, and $\mu = 10^{-4}$. Blue lines are theoretical predictions for the heterogeneous case: the mean $\bar{\tau}$ is calculated numerically from Eqs. (2) and (3) and $c_v = A\sqrt{M}/(M + B)$ with A and B fitted to data.

emerges is then

$$\bar{\tau} \approx \sum_{m=1}^{M-1} T_m^{\text{wave}} + \sum_{m=2}^{M-1} T_m^{\text{mut}}. \quad (3)$$

Note that we neglect the short time for the first cell of genotype M to occur after genotype $M - 1$ has reached its stationary value, and we assume strong selection, so that backward mutations do not fix. Equation (3) decomposes the time to resistance into the independent contributions of each wave of mutants. The value of $\bar{\tau}$ calculated from Eqs. (2) and (3) is in good quantitative agreement with our simulation results (black line in Fig. 2b). Since each successive emergence of a new genotype m can be modelled as a Poisson process, we expect that in a given experiment, the measured value of τ will be equal to a constant contribution $\sum_{m=1}^{M-1} T_m^{\text{wave}}$ plus the sum of $M - 2$ exponentially-distributed Poisson waiting times with means T_m^{mut} . For our choice of MICs and drug distribution, T_m^{mut} and T_m^{wave} are approximately independent of m for large m , because then the stationary waves $N_m^*(x)$ just differ by a constant spatial shift [42]. Hence for our model we expect τ to follow approximately a shifted Erlang distribution with the mean $\bar{\tau}$ growing linearly with the length of the pathway M , and its coefficient of variation $c_v = \sqrt{\tau^2 - \bar{\tau}^2}/\bar{\tau}$ scaling as $\sqrt{M}/(\text{const} + M)$. Figure 3 shows that this is indeed the case in our simulations, for pathways of length $M = 2, \dots, 20$. This prediction breaks down, however, for $M > m_{\text{crit}} \approx \alpha L / \ln(4) + 2 \approx 17$, because the population hits the right boundary before genotype M has evolved [48].

A different argument applies in the case where the drug is uniformly distributed, $c(x) = c$. For short pathways (small M), resistance evolves faster in the uniform environment than in the non-uniform one (Fig. 3, left panel), since the population size from which mutants can emerge is larger. However, beyond a critical pathway

length ($M > 4$ in our simulations), resistance emerges more slowly in the uniform environment, even though the population size is larger. In the uniform environment, new genotypes must establish in an already fully colonized space at fixed drug concentration. The time to fixation of genotype $m + 1$ is expected to scale with the fitness difference as $(\phi_{m+1} - \phi_m)^{-\gamma}$, where $\gamma > 0$ depends on whether mutations are rare or frequent [43]. In our model, the fitness advantage of genotype $m + 1$ over m is $\phi_{m+1} - \phi_m = c^2((1/c_m^{\text{mic}})^2 - (1/c_{m+1}^{\text{mic}})^2)$ which decreases for successive genotypes, because c_m^{mic} increases with m . Therefore, successive genotypes take longer to fix, leading to a super-linear increase of $\bar{\tau}(M)$ with M . This result is also valid for other functional forms of $\phi_m(c)$, as long as resistance increases with m . For long pathways, the scaling changes again: here, selection for successive mutants becomes so weak that backward mutations can establish and neutral drift dominates. The evolution can then be approximated by an unbiased random walk in genotype space; $\bar{\tau}$ is then given by the mean first passage time $0 \rightarrow M$ of a walker constrained to positive m , which scales as $\sim M^2$ [44], with coefficient of variation c_v independent of M (see Fig. 3, right panel). Note that here we have assumed that the MICs of intermediate genotypes remain fixed as M changes; one can also show [45] that the same general conclusions hold (including the heterogeneous case) if c_m^{mic} is scaled with M so as to keep the MIC of the most resistant genotype constant.

Fitness valley. We now contrast these results with the case where the pathway to resistance passes through a "fitness valley" - i.e. one of the intermediate genotypes m has a lower MIC (is less drug-resistant) than its neighbouring genotypes $m - 1$ and $m + 1$. This scenario can arise due to epistatic interactions between mutations [17, 18], such that two mutations are required to gain a particular fitness benefit. In particular, in our model we set $c_3^{\text{mic}} = 3.5$, keeping all the other $c_m^{\text{mic}} = 4^{m-1}$ as before, so that $c_2^{\text{mic}} > c_3^{\text{mic}} < c_4^{\text{mic}}$, as depicted in Fig. 2f. Figure 2d,e shows that the presence of the fitness valley has a dramatic effect on the time to resistance in the heterogeneous environment: $\bar{\tau}(\alpha)$ now rises steeply with α . Crucially, the shortest time to resistance in the heterogeneous environment is now comparable to that in the homogeneous environment, $\min_\alpha(\bar{\tau}(\alpha)) \approx \min_c(\bar{\tau}(c))$, and $\bar{\tau}(\alpha) > \min_c(\bar{\tau}(c))$ for almost all values of α . Thus, when the pathway to resistance contains a fitness valley, a non-uniform drug concentration does not speed up, and may well slow down the emergence of resistance.

To understand this effect, we argue that the rate-limiting step in the evolutionary process is the "tunnelling" through the fitness valley [46]: mutants of genotype 4 arise from the population of genotype 2 via short-lived mutants of genotype 3 which do not reach fixation. The tunnelling rate $\bar{\tau}^{-1}$ has been calculated for well-mixed populations in Ref. [46] (Eq. (2) therein). Applying this result to the case of uniform drug distribution we

obtain $\bar{\tau}^{-1} \approx rN_2(\mu/2)^2(P_{\text{fix}}/s)$ where $N_2 \approx LK(1 - d)$ is the population size of genotype 2, $s = (\phi_2 - \phi_3)/\phi_2$ is the selective advantage of genotype 2 over genotype 3, $P_{\text{fix}} = (\phi_4 - \phi_2)/\phi_2$ is the fixation probability of genotype 4 and r is the growth rate which in the steady state equals the death rate d . For our choice of $\phi(c)$ and c_m^{mic} , this gives

$$\bar{\tau} \approx 1.23/(d\mu^2 N_2) = 1.23/(d\mu^2 LK(1 - d)), \quad (4)$$

which is independent of c . Equation (4) is in good agreement with our simulation results (black line in Fig. 2d). Extending this approach to the heterogeneous case, we integrate over the steady-state population density of genotype 2:

$$\bar{\tau} \approx \frac{1.23}{d\mu^2} \left[\int_0^{x_2^*} N_2^*(x) dx \right]^{-1}. \quad (5)$$

This result agrees well with our simulation results for the non-uniform drug concentration (Fig. 2e). The increase in $\bar{\tau}$ with the steepness of the drug concentration profile α occurs because the domain occupied by population 2 decreases as α increases; the non-uniform drug concentration decreases the steady state population size of genotype 2, reducing the pool of cells from which mutants of genotype 4 can emerge and slowing down the evolution of resistance.

Conclusion. Our results show that the mutational pathway to drug resistance plays a crucial role in determining the effect of a spatial drug distribution on the time to evolve resistance. If fitness (i.e. level of drug resistance) increases monotonically along the mutational pathway, a non-uniform drug concentration can greatly accelerate the evolution of resistance, by a factor that increases dramatically with the length of the pathway. However, for short pathways, or those involving a fitness valley, a non-uniform drug concentration does not speed up the evolution of resistance - indeed, it may actually slow it down.

Our predictions can be verified experimentally. Recent microfluidic experiments have shown that gradients of the antibiotic ciprofloxacin greatly accelerate the emergence of resistance of the bacterium *E. coli* [16]. Although the mutational pathway in this case is not known, our results suggest that it is likely to be monotonic. Furthermore, we predict that repeating the experiments using cefotaxime (monotonic pathway [1]) should produce similar results, but that for streptomycin, which has a fitness valley [17, 18], resistance should emerge faster in a uniform drug concentration. This procedure could be generalized to infer the characteristics of unknown mutational pathways, by comparing the times to resistance of cells in a microfluidic device, for different drug concentrations and gradients.

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* Equal contribution

- [1] D. M. Weinreich, N. F. Delaney, M. A. DePristo, and D. L. Hartl, *Science* **312** 111 (2006).
- [2] E. Toprak et al., *Nature Genetics* **44** 101 (2012).
- [3] Y. C. Wang and M. Lipsitch, *Proc. Natl. Acad. Sci. USA* **103**, 9655 (2006).
- [4] C. T. Bergstrom, M. Lo, and M. Lipsitch, *Proc. Natl. Acad. Sci. USA* **101**, 13285 (2004).
- [5] S. Nissen-Meyer, *Biometrics*, **22** 761 (1966).
- [6] D. J. Austin, K. G. Kristinsson, and R. M. Anderson, *Proc. Natl. Acad. Sci. USA* **96** 1152 (1999).
- [7] M. Lipsitch and B. Levin, *Antimicrob. Agents and Chem.* **41** 363 (1997).
- [8] S. Bonhoeffer, M. Lipsitch, and B. R. Levin, *Proc. Natl. Acad. Sci. USA* **94** 12106 (1997).
- [9] R. Chait, A. Craney, and R. Kishony, *Nature* **446**, 668 (2007).
- [10] J. P. Torella, R. Chait, and R. Kishony, *PLoS* **6**, e1000796 (2010).
- [11] N. Jumbe et al., *J. Clinical Investigation* **112**, 275 (2003).
- [12] M. Müller, A. dela Pena, and H. Derendorf, *Antimicrob Agents Chemother.* **48**, 1441 (2004).
- [13] A. I. Minchinton and I. F. Tannock, *Nat. Rev. Cancer* **6**, 583 (2006).
- [14] P. S. Stewart, *J. Bacteriol.* **185**, 1485 (2003).
- [15] O. Trédan, C. M. Galmarini, K. Patel, and I. F. Tannock, *JNCI J. Natl. Cancer Inst.* **99**, 1441 (2007).
- [16] Q. Zhang et al., *Science* **333** 1764 (2011).
- [17] S. J. Schrag and V. Perrot, *Nature* **381**, 120 (1996).
- [18] S. J. Schrag, V. Perrot, and B. R. Levin, *Proceedings of the Royal Society of London. Series B: Biological Sciences* **264**, 1287 (1997).
- [19] D. M. Weinreich, R. A. Watson, and L. Chao, *Evolution* **59**, 1165 (2005).
- [20] M. Eigen, P. Schuster, *Naturwiss.* **64**, 541 (1977); C. O. Wilke, *BMC Evolutionary Biology* **5**, 44 (2005); M. A. Nowak, *Trends in Ecology and Evolution*, **7** (4), 118 (1992); K. Jain and J. Krug, in *Adaptation in Simple and Complex Fitness Landscapes*, p. 299 (Springer Berlin 2007); L. Demetrius, *J. Chem. Phys.* **87** (12), 6939 (1987); I. Leuthäusser, *J. Stat. Phys.* **48**, 343 (1987); E. Baake, M. Baake and H. Wagner, *Phys. Rev. Lett.* **78**, 559 (1997).
- [21] J. Franke, A. Klözer, J. A. de Visser, and J. Krug, *PLoS* **7**, e1002134 (2011).
- [22] N. Beerenwinkel et al., *PLoS Comput. Biol.* **3**, e225 (2007).
- [23] I. Bozic et al., *Proc. Natl. Acad. Sci.* **107**, 18545 (2010).
- [24] M. T. Gastner, B. Oborny, A. B. Ryabov, and B. Blasius, *Phys. Rev. Lett.* **106**, 128103 (2011).
- [25] K. D. Behrman, M. Kirkpatrick, *J. Evol. Biol.* **24**, 665 (2011).
- [26] M. Kirkpatrick and N. H. Barton, *Am. Nat.* **150**, 1 (1997).
- [27] M. Droza, A. Pekalski, *Physica A* **362** 504 (2006).
- [28] F. Mizera and G. Meszéna, *Evol. Ecol. Res.* **5** 1 (2003).
- [29] T. B. Kepler and A. S. Perelson, *Proc. Natl. Acad. Sci. USA* **95**, 11514 (1998).
- [30] J.-T. Kuhr, M. Leisner, and E. Frey, *New J. Phys.* **13** 113013 (2011).
- [31] E. A. Martens, R. Kostadinov, C. C. Maley, and O. Hallatschek, *New J. Phys.* **13**, 115014 (2011).
- [32] E. A. Martens, O. Hallatschek, *Genetics* **189**, 1045 (2011).
- [33] A. Ali, S. Grosskinsky, and E. Somfai arXiv:1111.2992.
- [34] T. Reichenbach, M. Mobilia, and E. Frey, *Phys. Rev. Lett.* **99**, 238105 (2007).
- [35] B. Waclaw, R. J. Allen, and M. R. Evans, *Phys. Rev. Lett.* **105**, 268101 (2010).
- [36] See supplemental video material at <http://www2.ph.ed.ac.uk/~bwaclaw/antibiotics/>
- [37] R. R. Regoes, C. Wiuff, R. M. Zappala, K. N. Garner, F. Baquero, and B. R. Levin, *Antimicrobial Agents and Chemotherapy* **48**, 3670 (2004).
- [38] See supplemental text material at <http://www2.ph.ed.ac.uk/~bwaclaw/antibiotics/>, Sec. I
- [39] See supplemental text material Sec. II
- [40] J.D. Murray, *Mathematical Biology*, 3rd edition, Vol.1, Springer (2002).
- [41] M. A. Nowak, *Evolutionary dynamics. Exploring the Equations of Life*, Belknap Press (2006).
- [42] See supplemental text material Sec. III
- [43] See supplemental text material Sec. IV
- [44] I. Goldhirsch and Y. Gefen, *Phys. Rev. A* **33**, 2583 (1986).
- [45] See supplemental text material Sec. V
- [46] D. M. Weinreich and L. Chao, *Evolution* **59**, 1175 (2005).
- [47] For long pathways or for higher mutation rates, mutants later in the pathway can coexist due to reduced selection pressure, see the movies [36]
- [48] In this case, there is no fitness advantage for genotypes with $m > m_{crit}$, evolution becomes neutral and $\bar{\tau}$ grows faster than linearly with M .